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Tetanus Toxoid carrier protein induced T-helper cell responses upon vaccination of middle-aged adults

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ABSTRACT

Introduction: Vaccines frequently induce suboptimal immune responses in the elderly, due to immunological ageing. Timely vaccination may be a strategy to overcome this problem, which classifies middle-aged adults as an interesting target group for future vaccine interventions. However, the immunological fitness of the middle-aged population is ill-defined. It is currently unknown whether effective T-cell help towards B-cells is initiated by conjugate-carrier vaccines at middle-age.
Aim: We characterized systemic Tetanus Toxoid (TT) specific T-helper cell responses in the circulation of middle-aged adults (50–65 years of age, n = 31) having received the MenACWY-TT vaccination.
Methods: Blood samples were taken pre- as well as 7 days, 28 days, and 1 year post-vaccination. TT-specific T-cell responses were determined by IFNγ Elispot and by the secretion of IFNγ, IL13, IL10, IL17, and IL21 in cell culture supernatants. Circulating CD4+CXCR5+ICOS+IL21+ cells were analyzed by flow cytometry, and meningococcal and TT-specific IgG responses by bead-based immunoassays. The correlation between the T-cell help and humoral responses was evaluated.
Results: Vaccination with a TT-carrier protein induced a mixed TT-specific Th1 (IFNγ), Th2 (IL13, IL10), and Th17 (IL17) response in most participants. Additionally, circulating CD4+CXCR5+ICOS+IL21+ cells were significantly increased 7 days post-vaccination. Pre-vaccination TT-specific cytokine production and post-vaccination Th2 responses correlated positively with the increase of CD4+CXCR5+ICOS+IL21+ cells. No correlation between T-cell help and antibody responses was found.
Conclusion: The characteristics of the T-cell response upon a TT-carrier vaccination suggests effective T-cell help towards B-cells in response to meningococcal polysaccharides, although the absence of a correlation with the antibody responses warrants further clarification. However, the robust T-helper cell response in middle-aged adults, decades after previous TT vaccinations, strengthens the classification of this age group for future vaccine interventions in the context of population ageing.

1. Introduction

Immunological ageing is characterized by clear compositional changes in the T-cell compartment [1–5], which become already apparent in the 6th decade of life [2]. This immunological ageing increases the susceptibility towards infectious diseases and reduces the immune response to vaccines [6]. Therefore, timely vaccination is essential to circumvent the impact of this so called 'immunosenescence' and thereby enhance memory immunity in the ageing population. Consequently, future vaccination programs need to be developed towards a life-course scheme [7–9]. From this perspective middle-aged adults are an interesting group for studying appropriate future vaccine programs. However, information about the immunological fitness and consequently vaccine responses in middle-aged individuals is lacking.

It is currently unknown whether conjugate-carrier vaccines are able to induce effective T-helper cell responses in middle-aged adults. In infants, carrier proteins are used to induce T-cell help to the B-cell epitopes in order to generate long-term protection and memory B-cell formation in response to bacterial polysaccharides [10]. Not only the strength but also the type of the T-helper cell response influences the quality of the T-cell help. In general Th2 responses provide more efficient B-cell help than do Th1

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responses, which may affect the functionality of the humoral response [11–14]. In addition, T-cell help is mainly provided by follicular helper T-cells (T_{FH}-cells) within the germinal centers (GCs), where the B- and T-cells interact [15,16]. With advancing age, altered numbers of these T_{FH}-cells, lower T_{FH}-cell functionality, and diminished formation of GCs were observed [17,18], possibly negatively affecting the T-cell help after conjugate-carrier vaccination in older age groups.

Interestingly, it was recently described that an ongoing B:T-cell interaction in the GCs is mirrored by higher frequencies of T_{FH}-cells in the circulation at 6 to 8 days post-vaccination [15,18–21]. Until now, no consensus on the exact phenotype of circulating T_{FH}-cells is established. Similar to GC T_{FH}-cells, circulating CXCR5+ cells were previously described to promote B-cell differentiation and antibody secretion [13,22–24]. A key characteristic of T_{FH}-cells is the production of interleukin 21 (IL21), a cytokine involved in GC development, Ig isotype switching, hyper-mutation of the IgG heavy chain, IgG affinity maturation, and memory B-cell formation [13,25–28]. Moreover, the inducible costimulatory molecule (ICOS) was found essential in the interaction of T_{FH}-cells with the follicular B-cells [17,29,30]. Finally, multiple studies described roles for programmed cell death 1 (PD1) in phenotype characterization of the peripheral T_{FH}-cells [13,15,18,20,22,31]. Although some studies suggest a direct link between the T_{FH}-cell and the humoral responses [18,20,32], this is currently unclear.

In this study, we determined the induced T-cell responses initiated by a TT-carrier protein in the circulation of middle-aged adults (50–65 years of age) having received the TT-conjugated MenACWY vaccine (Nimenrix), in order to evaluate the T-cell help provided to B-cells in response to the bacterial polysaccharides in this age group. This information is essential for the understanding of T-helper cell responses at older ages and adds to knowledge on the immunological fitness of middle-aged adults.

2. Materials and methods

2.1. Study subjects and blood sampling

Thirty-one relatively healthy middle-aged (50–65 years of age) participants were randomly selected from a larger cohort. All participants were intramuscularly vaccinated with one dose of the TT-conjugated MenACWY vaccine (Nimenrix, GlaxoSmithKline (GSK)). Potential participants were excluded based on the following criteria: antibiotic use of fever (>38 °C) within the last 14 days, serious diseases demanding immune suppressive medical treatment within the last 3 months, a known or suspected immune deficiency, a blood coagulation disorder, a neurologic disorder, administration of blood products in the past 6 months, serious surgery within the last 3 months, the use of hormone supplementation, pregnancy, a suspected allergy towards the vaccine components, history of serious adverse events after previous vaccinations, a previous meningococcal vaccination, a previous meningococcal episode, a tetanus vaccination within the last 5 years, and any vaccination in the month before enrolment. Additional health characteristics of these participants are presented in Supplementary Table 1.

The Medical Ethical Committee: Medical Research Ethics Committees United (mec-U) (NTR4636) approved the study and written informed consent was obtained from all participants prior to the study. All procedures were in accordance with the Declaration of Helsinki.

Blood samples were taken pre-, as well as 7 days, 28 days, and 1 year post-vaccination. Peripheral blood mononuclear cells (PBMCs) were isolated using Vacutainer cell preparation tubes (CPT) containing sodium citrate (Becton Dickinson (BD)), according to the manufacturer's prescriptions. Cells were washed with RPMI-1640 medium (Gibco) supplemented with 1% heat inactivated fetal calf serum (FCS, Gibco), 1% Penicillin and Streptomycin (Lonza). PBMCs were counted and $7.5 * 10^6$ cells/mL were frozen in a 90% FCS 10% DMSO solution at −135 °C until further use. Serum was collected using serum clotting tubes (BD) and was stored at −20 until use.

From the initial cohort, PBMC samples obtained in sufficient quantities at all 4 time points were available from 31 participants for analysis. TT-specific T-cell responses were determined in 22 participants, whereas T_{FH}-cell markers were investigated in 20 participants. Samples from 11 participants provided enough cells for analysis in all assays (Fig. 1).

2.2. Tetanus specific T-cell stimulation and IFNγ Elispot

PBMC stimulation, supernatant collection and the IFNγ Elispot method were consecutively performed as previously described [33]. Based on prior titration and kinetics experiments, $3 * 10^5$ PBMCs/well were stimulated for 5 days with medium containing 5% AB serum (Sigma Aldrich) only (negative control), or with addition of 6.67 Lf/mL purified tetanus toxoid (NVI Bilthoven, The Netherlands) or 5 μg/mL Pokeweed mitogen (Sigma) as a positive control, in triplicate. Thereafter, stimulated cells were transferred to anti-IFNγ-coated Elispot plates (Mabtech) for 18 h. Elispot plates were scanned with the Epson ELISPOT Scanner and spots enumerated with the AELVIS software, using a standardized protocol. Antigen specific spots were calculated by subtraction of the spots in the negative controls. In order to avoid influences of assay variability, all 4 samples (pre-, 7 d, 28 d, and 1 year) of the study participants were measured in the same experiment employing the same batch of IFNγ coated Elispot plates.

2.3. Cytokine detection in supernatants

After 5 days of PBMC stimulation, cell culture supernatants were collected and stored at −80 °C until further use. Subsequently, IFNγ, IL13, IL10, IL17, and IL21 concentrations in the supernatants were determined by commercially available multiplex bead-based immunoassay kits according to the manufacturer's instructions (Bio-Rad Laboratories). Samples with concentrations below the lower limit of quantification were assigned half the concentration of the lowest measurement. Cytokine concentrations in the negative controls were subtracted from those in the antigen stimulated samples.

2.4. Detection of CD4+CXCR5+ICOS+IL21+ cells by flow cytometry

To assess the potency of circulating T-cells to produce IL21, $3 * 10^5$ PBMCs/well of samples pre- as well as 7 and 28 days post-vaccination were incubated with medium only (negative control) or with a cell stimulation cocktail containing phorbol 12-myristate 13-acetate (PMA) and ionomycin (IONO) in a suboptimal 2000× dilution (eBiosciences) in triplicate. Cells were incubated for 6 h at 37 °C with 5% CO_2. After one hour, GolgiPlug protein transport inhibitor containing Brefeldin A (1000× dilution, BD) was added to each well. After thorough washing, cells were incubated for 30 min with a mixture of Life-Death Zombie Aqua fluorescent dye (Biolegend) and surface antibodies in FACS buffer, containing PBS with 0.5%BSA and 2 mM EDTA. The following antibodies were used for the surface staining: CD3 (UCHT1) – V450, CD4 (RPA-T4) – APC, CXCR5 (RF8B2) – PerCP-Cy5.5, PD1 (MIH4) – FITC (all BD), and ICOS (C398.4A) – APC-Cy7 (Biolegend). Thereafter, cells were washed with PBS, and permeabilized for 20 min with Cytofix/Cytoperm (BD). A Perm/Wash solution (BD) was used to wash the cells. In addition, the cells were stained for 30 min

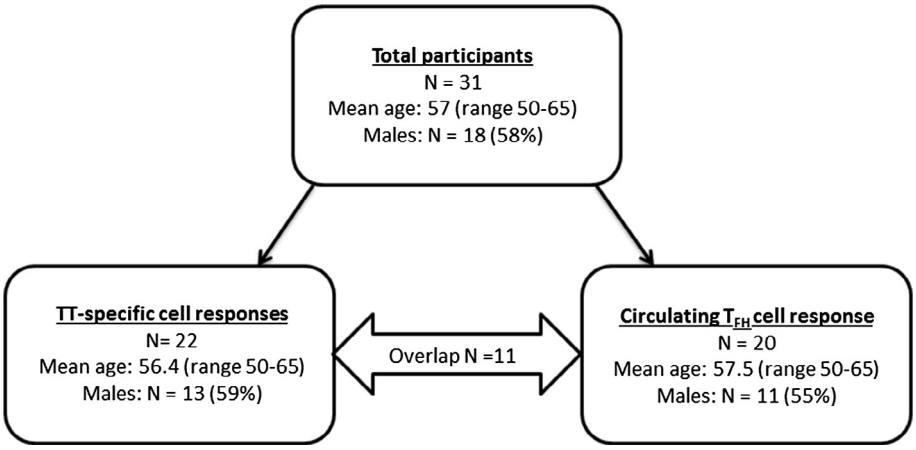

Fig. 1. Participants flow chart.

with IL21 (3A3-N2.1)-PE (BD) and with CD4 (RPA-T4)-APC detecting intracellular IL21 and CD4, respectively. After washing, the cells were resuspended in cold FACS-fixation buffer (Biolegend) until measurement. Flowcytometry analysis was performed on a 4-lazer LSR Fortessa (BD) and data was analyzed using FlowJo V10.

2.5. Serological analysis

Tetanus and meningococcal specific IgG concentrations were determined using the fluorescent-bead-based multiplex immunoassay (MIA) as previously described [34,35].

2.6. Statistics

The number of TT-specific IFNγ spots, cytokine concentrations, CD4+CXCR5+ICOS+IL21+ cell responses, and the IgG responses at the different time points were compared with the Wilcoxon signed rank test, preceded by the Friedman test. Correlations were determined by the Spearman's rho correlation test. Graphpad V7 and SPSS V22.0 were used.

3. Results

3.1. TT-specific IFNy-producing cells are boosted after vaccination

Numbers of TT-specific IFNγ-producing T-cells were enumerated pre- and post-vaccination (Fig. 2). Pre-vaccination, already high numbers (>10 spots/10^5 PBMCs) of TT-specific IFNγ-producing cells were found in the majority of the participants (16 out of 22). These numbers significantly increased 28 days post-vaccination; a strong trend was seen after 7 days. One year post-vaccination, the numbers of TT-specific IFNγ-producing cells returned to pre-vaccination values (Fig. 2a). Although the response was highly variable (Fig. 2b), most participants showed an enhanced IFNγ T-cell response either at day 7 or at day 28 post-vaccination, indicating that vaccination with a TT-carrier protein boosted a short-term Th1 type response in middle-aged adults.

3.2. Vaccination with a TT-carrier protein boosted mixed T-helper cytokine responses

Next, we analyzed TT-specific Th1 (INFγ), Th2 (IL10, IL13), Th17 (IL17) and T_{FH} (IL21) cytokine secretion profiles. Similar to the numbers of IFNγ-producing cells, TT vaccination led to increased TT-specific IFNγ production at days 7 and 28 post-vaccination (Fig. 3a). Also, a marked increase of the IL13 production was seen post-vaccination (Fig. 3b), which resulted in a relative decrease of the TT-specific IFNγ/IL13 ratio at 28 days post-vaccination (Fig. 3c).

Additionally, TT-specific IL10 production was largely enhanced 7 days post-vaccination in all participants, after which this response gradually declined 28 days post-vaccination (Fig. 3d). Moreover, a significant but limited enhancement of TT-specific IL17 secretion was observed both at day 7 and 28 (Fig. 3e). Of note, TT-specific IL21 production was below detection limit (data not shown). Overall, 7 days post-vaccination, a mixed cytokine profile was induced in

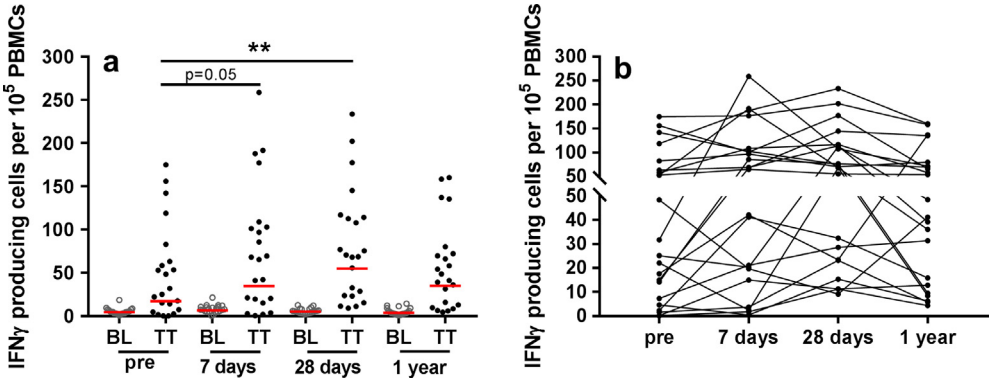

Fig. 2. Numbers of TT-specific IFNγ producing cells. (a) The numbers of IFNγ producing cells/10^5 PBMCs pre- and post-vaccination in the negative control (BL) and after stimulation with Tetanus Toxoid (TT). The horizontal line indicates the geometric mean concentration. (b) Paired numbers of IFNγ producing cells/10^5 PBMCs pre- and post-vaccination. The different time points were compared with the Wilcoxon signed rank test preceded by the Friedman test. $^{**}p < 0.01$, n = 22.

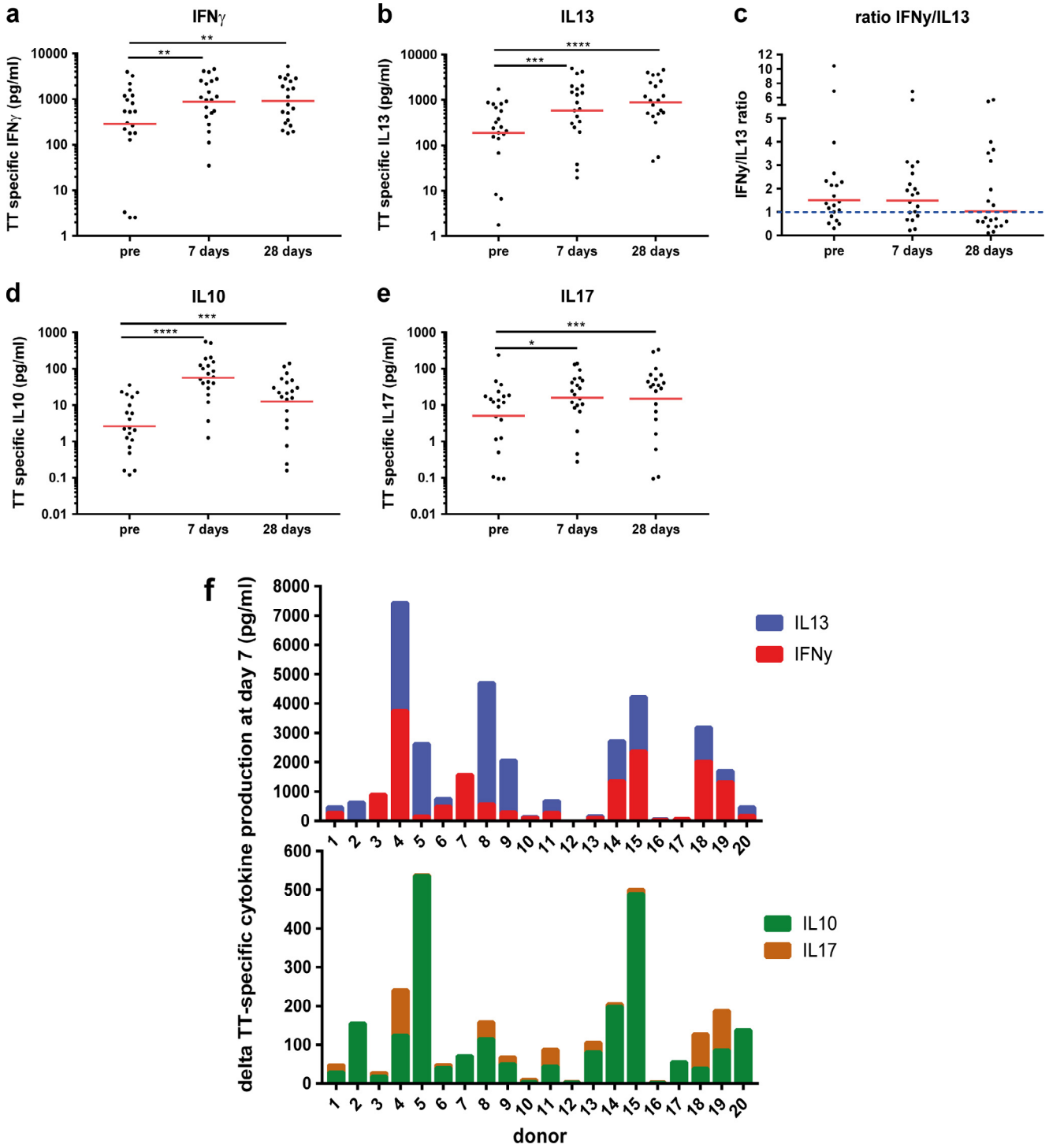

Fig. 3. TT-specific cytokine responses. Concentrations (pg/mL) of IFNγ (a), IL13 (b), IL10 (d), and IL17 (e) in cell culture supernatants after 5 days of Tetanus Toxoid stimulation. The red line indicates the geometric mean concentration. (c) The IFNγ/IL13 ratio pre- and post- vaccination. The different time points were compared with the Wilcoxon signed rank test preceded by the Friedman test. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. n = 20. (f) The delta TT-specific cytokine responses at day 7 (day 7 – day 0) per donor.

most donors, although some participants clearly showed a Th1 (IFNy) or Th2 (IL10, IL13) dominated response (Fig. 3f).

3.3. The TT-carrier protein induced circulating CD4+CXCR5+ICOS+IL21 + cells

Although we did not detect TT-specific IL21 production, we next investigated the induction of circulating CD4+CXCR5+ICOS+IL21+ cells following vaccination by TT-specific or polyclonal stimulation with PMA/IONO (Fig. 4a). Since PMA/IONO stimulation caused the internalization of CD4 by T-cells, both intra- and extra-cellular staining of CD4 was performed to detect the total CD4 T-cell pool. Firstly, no intracellular IL21 production was observed after TT-specific stimulation (data not shown). Stimulation with PMA/IONO induced IL21 production in both the CXCR5+ and CXCR5- subsets, although this production was significantly higher in the CXCR5+

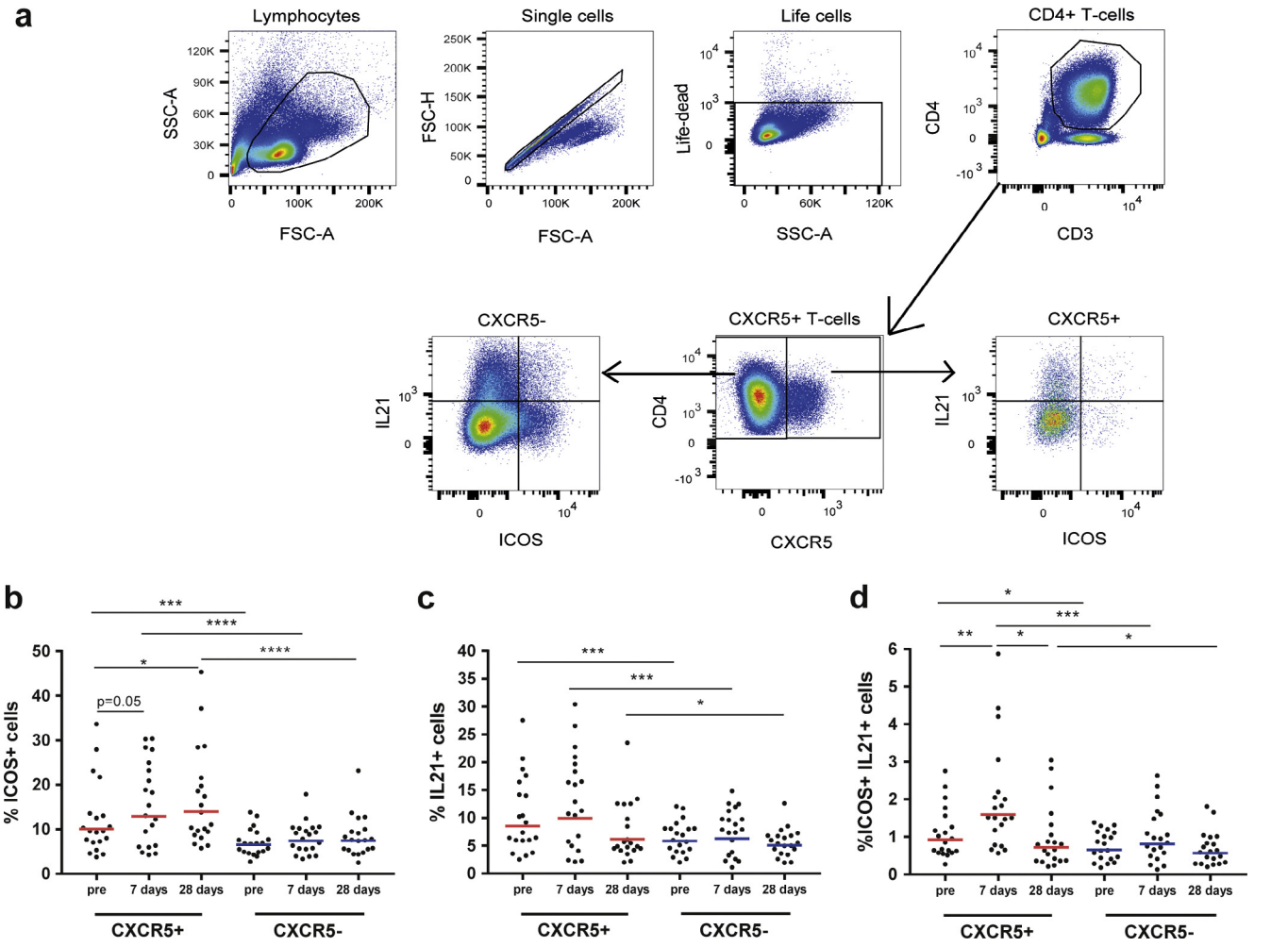

Fig. 4. Induction of circulating CD4+CXCR5+ICOS+IL21+ cells post-vaccination. (a) Gating strategies used for characterization of the CD4+CXCR5+ICOS+IL21+ cells. A representative sample is shown. (b) Percentages of ICOS+ cells within the CXCR5+ and CXCR5- populations pre- and post-vaccination (7 and 28 days). (c) Percentages of IL21+ cells within the CXCR5+ and CXCR5- populations pre- and post-vaccination (7 and 28 days). (d) Percentages of ICOS+IL21+cells within the CXCR5+ and CXCR5- populations pre- and post-vaccination (7 and 28 days). The horizontal lines in the graph represent the geometric mean percentages. The different time points were compared with the Wilcoxon signed rank test preceded by the Friedman test. CXCR5+ and CXCR5- cells at the different time points were compared with the Mann Whitney *U* test. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, n = 20.

cells (Fig. 4c). Also, ICOS expression was higher in the CXCR5+ cells (Fig. 4b). Similarly, higher frequencies of IL21+ICOS+ cells were detected in the CXCR5+ subset (Fig. 4d). Seven days post vaccination a significant increase in the frequency of IL21+ICOS+ cells within the CXCR5+ compartment was observed (Fig. 4d). This response was variable between the participants, with 17 responders and 3 non-responders. Additionally, some samples (n = 12) were analyzed for PD1 expression (Supplementary Fig. 1b). A strong significant correlation between the percentages of CXCR5+IL21+ICOS+ cells and CXCR5+IL21+ICOS+PD1+ cells 7 days post-vaccination was found (Supplementary Fig. 1c). These results indicate a significant increase in activated T-cells that have high potential for B-cell help after vaccination with a TT-carrier protein in middle-aged adults.

3.4. TT-specific T-helper cell cytokines correlate with the CD4+CXCR5+ICOS+IL21+ cell response 7 days post-vaccination

We further assessed whether the increase in the CD4+CXCR5+ICOS+IL21+ cells at 7 days post-vaccination correlated with TT-specific T-helper cytokine production, both pre- and post-vaccination. Seven days post-vaccination, a strong correlation was observed between the increase in CD4+CXCR5+ICOS+IL21+ cells and the increase in TT-specific IL10 and IL13 production (Table 1a, sub 1). The increase in CD4+CXCR5+ICOS+IL21+ cells did not correlate with the TT-specific Th17 or Th1 response, although a trend was seen for the latter.

Next we analyzed the correlation between pre-vaccination TT-specific cytokine production and the increase in CD4+CXCR5+ICOS+IL21+ cells at day 7. A significant correlation was found between the production of pre-vaccination TT-specific IFNγ and the increase in CD4+CXCR5+ICOS+IL21+ cells 7 days post-vaccination (Table 1a, sub 2). Moreover, a positive trend was found for the other T-helper subsets, suggesting that the presence of pre-vaccination TT-specific T-helper cells enhanced the CD4+CXCR5+ICOS+IL21+ cells responses.

3.5. TT-specific T-helper cell responses do not correlate with antigen specific IgG responses

The TT-conjugated meningococcal vaccine induced clear antibody responses to TT and the meningococcal C polysaccharides (MenC-PS) (Fig. 5). Yet, no correlations between the TT-specific T-helper cell responses or the CD4+CXCR5+ICOS+IL21+ cells

Table 1a
Correlations between the TT-specific cytokine responses and the induction of circulating CD4+CXCR5+ICOS+IL21+ cells.

Parameter	variable	Spearman's rho	p-value
1. Delta TT-specific cytokine response at day 7	IFNγ	0.650	0.058
(day 7 – day 0, pg/ml)	IL13	0.700	**0.036**
	IL10	0.850	**0.004**
	IL17	0.367	0.332
2. Pre-vaccination TT-specific cytokine response	IFNγ	0.795	**0.010**
(pg/ml)	IL13	0.503	0.138
	IL10	0.661	0.053
	IL17	0.571	0.084

The delta % CD4+CXCR5+ICOS+IL21+ cells at days 7 (7 days – pre-vaccination) was used in the correlations. n = 10. The Spearman's rho correlation test was used. The p-values of significant ($p < 0.05$) correlations are indicated in bold.

Table 1b
Correlations between the induced circulating CD4+CXCR5+ICOS+IL21+ cell response, TT-specific cytokine responses and the TT and MenC-PS specific IgG responses.

T1-T0 response	parameter	Time point	Spearman's rho	p-value
CXCR5+ICOS+IL21+	TT IgG	28 days	−0.135	0.569
	TT IgG	1 year	−0.271	0.248
	MenC IgG	28 days	0.056	0.816
	MenC IgG	1 year	0.039	0.870
IL10	TT IgG	28 days	0.168	0.478
	TT IgG	1 year	0.026	0.915
	MenC IgG	28 days	0.194	0.413
	MenC IgG	1 year	0.206	0.384
IL13	TT IgG	28 days	0.265	0.259
	TT IgG	1 year	0.277	0.238
	MenC IgG	28 days	0.152	0.523
	MenC IgG	1 year	0.217	0.359
IFNγ	TT IgG	28 days	0.245	0.298
	TT IgG	1 year	0.084	0.724
	MenC IgG	28 days	0.314	0.177
	MenC IgG	1 year	0.307	0.188
IL17	TT IgG	28 days	0.198	0.402
	TT IgG	1 year	0.176	0.458
	MenC IgG	28 days	−0.060	0.801
	MenC IgG	1 year	0.048	0.840

The delta % of CD4+CXCR5+ICOS+IL21+ cells at days 7 (7 days – pre-vaccination) was used in the correlations, as well as the delta cytokine production (7 days – pre-vaccination). n = 20. The Spearman's rho correlation test was used.

response with the MenC PS-specific and TT-specific IgG responses were detected, using delta (Table 1b), absolute, and fold change T-cell responses (data not shown). On a side note, positive correlations were found between the pre- and post-vaccination IgG concentrations for TT (7d: rho: 0.591 p = 0.006; 28 d: rho: 0.711 p = <0.0001, 1y: rho: 0.713 p < 0.0001), but not for MenC. Adjusting for pre-vaccination immunity did not change our results. Similar results were obtained for the other meningococcal serotypes, and for the IgG subclass responses (data not shown).

4. Discussion

Within this study, we show that vaccination of middle-aged adults with a TT-carrier protein, used to initiate T-cell help in response to meningococcal polysaccharides, induced a mixed Th1, Th2, and Th17 T-cell response, although some participants showed a clear Th1 (IFNy) or Th2 (IL10, IL13) dominated response. In addition, the enhanced frequencies of circulating CD4+CXCR5+ICOS+IL21+ cells 7 days post-vaccination suggests the induction of activated T-cells with T_{FH} cells properties by a TT-carrier protein in older adults. These T-helper cell responses are suggestive of effective T-cell help towards the B-cells in response to the meningococcal polysaccharides, although we did not find a direct correlation between the T-cell response induced by the carrier protein and the meningococcal specific antibody responses.

Although a mixed cytokine response was observed, our data suggests a slightly skewed Th2 response post-vaccination, mainly represented by a strong IL10 response 7 days post-vaccination and a decreased post-vaccination IFNy/IL13 ratio. The strong IL10 response may both regulate the Th1 response [36], and stimulate the antibody responses towards the bacterial polysaccharides [12,13]. This positive effect of IL10 was strengthened by the strong positive correlation between the IL10 production and the circulating CD4+CXCR5+ICOS+IL21+ cell response at day 7 post-vaccination.

Antigen specific detection of IL21 production by T-cells is notoriously difficult, since B-cells directly consume secreted IL21 and the T_{FH}-cells need vigorous *in vitro* stimulation to produce IL21 [31,37,38]. Therefore, we assessed the potency of IL21 production by T-cells following PMA/IONO stimulation and intercellular detection of IL21. Indeed, IL21+ cells were found in both the CXCR5+ and CXCR5- subset, as was also shown by others [22,23,31]. Yet, higher frequencies of IL21+ cells were observed in the CXCR5+ subset as compared to the CXCR5- counterpart, which is in agreement with others [23]. Although it was previously shown that IL21 production of both subsets provided help towards B-cells, this was significantly increased in CXCR5+ cells due to the elevated expression of ICOS, a B-cell co-stimulator [22]. Therefore, IL21 production alone is perceived insufficient to determine efficient T_{FH}-cell responses [22,31]. Post-vaccination, we observed a tendency towards increased expression of ICOS in the CXCR5+ subset (data not shown), which is in line with findings by Herati et al. after influenza vaccination in the elderly [18]. Taken together, our findings of increased frequencies of CXCR5+ICOS+IL21+ cells 7 days

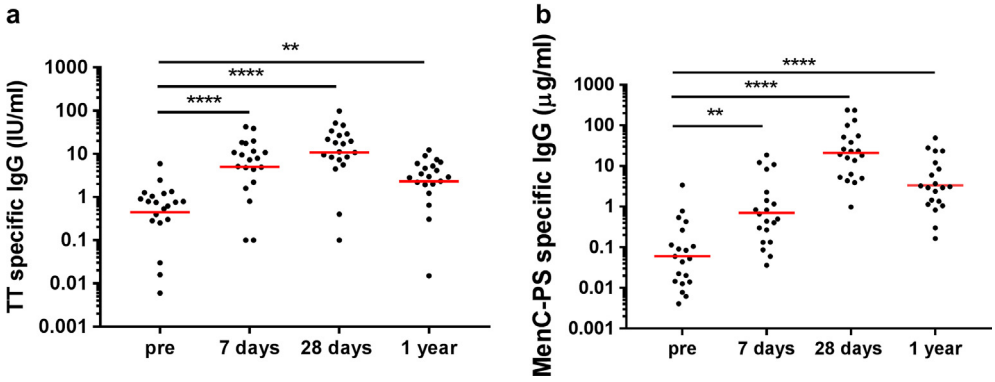

Fig. 5. Anti TT and MenC-PS specific IgG responses. TT (a) and MenC-PS (b) specific IgG responses pre-, and post- vaccination. The different time points were compared with the Wilcoxon signed rank test preceded by the Friedman test. $^{**}p < 0.01$, $^{****}p < 0.0001$, n = 20.

post-vaccination suggest the induction of T_{FH}-cells. This was strengthened by the strong correlation between the frequencies of CXCR5+ICOS+IL21+ cells and the CXCR5+ICOS+IL21+PD1+ cells 7 days post-vaccination, since circulating PD1+ T_{FH} cells share functional properties with GC T_{FH} cells [20,23,32].

In addition, we observed a correlation between the increased frequencies of CD4+CXCR5+ICOS+IL21+ cells at day 7 and the presence of TT-specific pre-vaccination cytokine production, indicating that pre-vaccination T-cell immunity enhances T-cell responses upon conjugate-carrier vaccination. Although only 3 of the participants received a TT vaccination after the year 2000, pre-vaccination TT-specific T-cell cytokine production was observed in most participants, which indicates that TT-specific T-helper cells persist for years or even decades after historical vaccinations in most participants. However, due to the exclusion of individuals with recent (<5 years) TT vaccinations in this study, the effects of recent tetanus vaccinations on the T-cell response towards the TT-carrier protein remains unknown. Of note, pre-vaccination TT-specific IgG concentrations did not correlate with TT-specific cytokine production and thus cannot be used as surrogate marker for the presence of TT-specific T-cell immunity.

In spite of the induction of T-cell responses that favour B-cell help by the TT-carrier protein, we did not observe an association between the strength of the T-cell response and the TT or meningococcal polysaccharide specific antibody concentrations. The absence of this correlation might be explained by several factors. First of all, the multivalent nature of the meningococcal vaccination might complicate the detection of a correlation between the T-cell and the humoral response. Moreover, the different polysaccharide antigens induced diverse antibody responses within the same person (data not shown). Therefore, we suggest a large effect of the existing B-cell repertoire in response to the polysaccharide antigens, a notion corroborated by the findings of other studies [28,39]. Additionally, the lack of a correlation between the T-helper cell and antibody responses at the time points analysed may be explained by potential differences in peripheral B- and T-cell kinetics. Furthermore, we propose that simply the presence but not the strength of the T-cell response is sufficient to induce an effective vaccine antibody response, also that is described for a conjugated meningococcal vaccination in infants [40]. Finally, although our results might suggest the induction of circulating activated T-cells with T_{FH} cell properties after vaccination, the exact functionality of these CD4+CXCR5+ICOS+IL21+ cells is currently unknown. In particular the relation of these CD4+CXCR5+ICOS+IL21+ cells to a possible T_{FH} cell response in the GCs, clearly awaits further confirmation, especially in relation to immunological ageing, since GC and T_{FH}-cell functionality might be altered with advancing age [17,41]. It has been described that circulating T_{FH}-cells did correlate with influenza specific antibody responses post-vaccination in young adults but not in the elderly [18], this might indicate that GC responses are diminished in older adults although circulating T_{FH} cells are present.

In this study, we did not detect clear age related differences in the T-cell responses, which might be influenced by the limited age range, small sample size in our study, and possible confounding effects of pre-vaccination T-cell immunity.

In conclusion, vaccination with a TT-carrier protein in middle-aged adults induced a mixed TT-specific T-helper cell response. Moreover, the induction of circulating CD4+CXCR5+ICOS+IL21+ cells 7 days post-vaccination suggests a circulating T-cell response with high potential for B-cell help. Subsequently, the characteristics of these T-cells proposes effective T-cell help towards B-cells in response to the meningococcal polysaccharides, although a direct correlation with the antibody responses was not found, a finding that warrants further clarification especially in the context of immune ageing. Notwithstanding, the robust T-helper cell response, decades after previous TT vaccination, adds to the knowledge on the immunological fitness of middle-aged adults. Moreover, these results strengthen the classification of this age group as an interesting target group for future vaccine interventions, to stimulate memory immunity before reaching old age.

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Author contributions

MvdH, AD, GAMB, AMHB and A-MB designed the experiments. MvdH planned and performed the clinical work. MvdH and AD executed the laboratory experiments. MvdH, AD, AMH, and A-MB analysed and interpreted the data. MvdH, GAMB, AMHB, and A-MB wrote the manuscript. All authors critically revised the manuscript.

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Conflict of interest

MvdH, AD, GAMB, and A-MB declare no conflict of interest. AMBH is a consultant for Grunenthal Gmbh (Germany) and was formerly employed (until October 2011) by MSD (Merck Research Laboratories in Oss, The Netherlands).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2017.08.056.

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